THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

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REPRINTED FROM
THE ANNALS OF APPLIED BIOLOGY
VOL. XVII, NO. 3, AUGUST, 1930

CAMBRIDGE
AT THE UNIVERSITY PRESS
PRINTED IN GREAT BRITAIN

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Harpenden, Herts
England

[From THE ANNALS OF APPLIED BIOLOGY, Vol. XVII, No. 3, August, 1930.]

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THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

I. THE MOVEMENT OF MOSAIC IN THE TOMATO PLANT

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(With Plate XXXIII.)

Introduction.

The study of virus diseases has, in the last few years, become of great importance. It has been increasingly recognised that many of the common diseases both of plants and of animals are attributable to agents called filter-passing viruses. At present, the main criteria of a virus disease are the symptom-complex induced, its infectivity and the invisibility of the causative agent. The structure of the causative agent is, in the nature of things, not at present recognisable. The general position of virus diseases has been discussed recently by various authors, and reference may be made to their papers for details.

Recently attempts have been made to assess the physical characters (resistance to heat, to alcohol, etc.) of the infective principle and a beginning has been made with the study of the physiological aspects.

As virus diseases are recognisable mainly by their symptoms and their infectivity, these are the two aspects of the problem which have been most studied under laboratory conditions. It is clear, however, that symptoms do not serve as very reliable indices of this type of disease on account of the inherent difficulty of rigidly defining a disease by symptoms which may, under differing environmental conditions, vary between wide limits.

As this uncertainty constitutes a real difficulty, it was decided to attempt, in this laboratory, some more general studies on the physiology of virus diseased plants. Various lines have been opened up, and the one with which this paper deals is that of the movement of the causative agent within the plant. This is an aspect which has been little studied. It was felt, however, to be of considerable importance in that it may be possible to give some indication of the nature of the causative agent if it be definitely shown how its movement through the plant takes place.

RESULTS OF PREVIOUS WORK ON THE MOVEMENT OF VIRUS.

The few data relating to this problem are rather scattered through the literature. Bennett (2) was one of the first workers to examine the actual tissues through which movement takes place. He investigated the movement of the curl virus of raspberries through "ringed" stems. From his experiments he concluded that (1) the curl virus can be confined to the inoculated shoot for an indefinite period by the simple process of "ringing," (2) a relatively small amount of "bark" bridging a "ring" is sufficient to permit of the passage of the curl virus from the inoculated part to the parts below the "ring." He suggested that the movement of the virus from the root of the resting stool "may parallel the movement of food."

Some time earlier Severin (14) had attempted to determine the rate of movement of the infective agent. He found that the virus of curly-top of sugar beet moved in "one-half hour at a mean temperature of 103·5° F. through the petiole 7 inches long." This was the quickest rate he obtained. More usually the rate was of the order of 4 inches per hour in his experiments. It was also found that the disease was transmitted from infected adults of *Eutettix tenella* feeding on the outer leaves of a plant to non-infected individuals on the inner leaves of the plant in 6 days. When the positions of the leaf-hoppers were reversed, the movement outwards took 10 days, at a slightly lower temperature.

The infective agent of curly-top was transmitted from infected hoppers on one of the first two leaves of a young beet plant to non-infected males on the opposite outer leaf at the end of 2 days at 81° F.

McCubbin and Smith (11) inoculated the main stem of tomato plants, the lower branches of which had been "layered" so as to induce rooting. By separating the daughter-plants after various intervals of time, they found that the virus agent travelled from 8 to 18 inches in 10–20 days. The virus agent apparently left the main plant between the 3rd and the 10th days.

Priode (12) found that, in the ringspot disease of tobacco, lesions frequently appear in the region of the inoculated leaf. Usually systemic infection follows. Holmes, in two papers (8, 9), has shown that the virus of tobacco mosaic may be found subsequent to inoculation above the inoculated leaf before it appears below. He has found that inoculation through broken trichomes takes place instantaneously, and that immediate washing off of the infected juice is not sufficient to prevent infection. Rubbing the infected juice on to wounds made some time before did not result in the appearance of symptoms.

Storey (17) found that the movement of the virus of the mosaic disease of maize across or down the leaf was not obviously impeded by cutting out portions of the midrib or by severing the veins of the half-lamina in which inoculation took place. "This result," he writes, "was to have been expected, for in either case the leaf remained turgid, being supplied by the small anastomosing veins which form a network through the leaf lamina." He found that movement down a leaf, subsequent to insect infection, was at a rate varying in six cases out of sixteen from 10 cm. to 20 cm. per hour.

Böning (4) found that the virus of mosaic disease travelled in tobacco leaf a distance of 13 cm. in a minimal period of 2 days and in tomato leaf a distance of 9 cm. in 2 days. In the tomato leaf "streak" travelled a shorter distance in 4 days. His results for rate of movement up and down the stem may be summarised in the following way. The results refer to tomato stems.

Table I.

Movement of virus agents in tomato stem (from Böning).

Mosaic.			*Streak.*		
Direction	Distance (cm.)	Time (days)	Direction	Distance (cm.)	Time (days)
Down stem	12	4	Down stem	12	6–7
Down stem	20	4–5	Down stem	20	4–5
Up stem	12	3–4	Up stem	12	4–5
Up stem	25	4–5	Up stem	25	5

It will be seen that there is some variation in the results, but they all do definitely suggest that the movement of the virus is comparatively slow, and that it moves in either direction in the plant at rates of the same order of magnitude.

Recently, Davis (7) has summarised the literature on the Infectious Chlorosis of Variegated Plants. He deals especially with the work of Baur and of Lindemuth. Baur found that the causative agent of chlorosis in *Abutilon avicennia* apparently moves through the extra cambial tissues. He prevented the movement by "ringing" the stem. He also suggested that the agent could move through an immune *A. arboreum* stem grafted on an *A. avicennia* without multiplication—the former stem being a mechanical means of transport. On the other hand, Blakeslee (3) has shown that the agent of the "Q" disease of Datura did not travel through a Petunia stock on an infected scion.

THE PROBLEM HERE TREATED.

The evidence adduced from the experiments on the rate of movement gives one but little indication of the tissues in which the movement is taking place. It was proposed, therefore, to deal with that aspect of the problem.

Various general considerations operated in suggesting the lines along which work might be carried out. It has already been shown by various authors (Auchter (1), Curtis (6), Caldwell (5)) that, when substances moved in the phloem or in the xylem either upwards or downwards in the plant, movement was considerably more free in a vertical than in a lateral direction. The movement laterally was much less rapid, if, indeed, in some cases it took place at all.

The first experiments were, therefore, set up with a view to studying localisation of the movement of the virus in the tissues of the plant. When an eosin solution was absorbed by a petiole stump, subsequent to the removal of the lamina, the eosin was found to travel upwards and downwards on the same side, and to pass over to the other side of the plant only after it had travelled out to and round the anastomosing vessels at the leaf-tips. In plants with decussate phyllotaxis the localisation was particularly well marked (see Caldwell (5)). A simple analogy suggested itself with the substitution of virus inoculum for the eosin solution. All the experiments hereafter described were carried out using the "aucuba" disease of tomatoes—a mosaic disease which has been described in detail by Henderson Smith (15). This disease was chosen for the work in this investigation because it is easily transmitted by juice inoculation, is very infectious, and shows particularly well-defined symptoms. The symptom-complex has already been discussed by Henderson Smith, who described the main features, in bright weather under normal conditions, as follows: "Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf." This disease is so infectious that it is easily transmitted merely by rubbing a healthy plant after infected plants have been handled. This feature is especially valuable when dealing with experiments yielding negative results, as, in the main, symptoms regularly follow inoculation.

Inoculations were made as follows: leaves from a plant showing aucuba symptoms were cut into small pieces and crushed in a mortar. Thereafter was added a volume of distilled water equivalent to twice the

weight of the leaf material. The whole was carefully pulped and mixed. A few drops of this material were transferred by means of a Pasteur pipette to the back of a marking label. This was held under the pinna to be inoculated, and another drop was placed on the upper surface. This drop was carefully spread over the whole of the upper side of the leaf. Some 30–40 holes were then pricked in the lamina—care being taken to distribute them as evenly as possible. In all cases where the plant as a whole was to be infected, four pinnae were treated, but in some cases—as when unilateral infection was attempted, only two pinnae (both on the same leaf) were so treated. All the apparatus used was sterilised so far as was possible, and the whole operation was carried out with the minimal chances of accidental infection.

INOCULATION ON ONE SIDE.

It was thought from analogy with the movement of metabolites that, if the movement of the virus agent was localised in the vascular elements, the tendency would be for symptoms to appear in the region of the treated shoot or leaf. Especially should the leaves on the same orthostichy show symptoms rapidly. As has before been pointed out, the axillary shoot has similar vascular connections to the leaf in the axil of which it develops. In the main, symptoms appear first on the younger leaves which are, naturally, at the top of the plant. As the axillary buds develop, however, their leaves also show symptoms.

In the first experimental plant C_2 inoculation was made on July 8th on the leaves of a single branch. Symptoms appeared at the top of the plant and on the leaves of the axillary bud above the treated shoot on July 21st. Two other shoots on the same side showed symptoms by July 22nd. The symptoms appeared on the leaves of the opposite axillary shoots on July 26th. There was in this case, therefore, some delay in the appearance of the symptoms on the side away from the inoculation.

In the other plants, C_1, C_3, inoculated at the same time and on leaves of a single branch, the symptoms appeared first at the top and then downwards as the axillary bud-leaves grew without any apparent localisation. Similar results were obtained with a fourth plant, C_9, which was inoculated on July 18th. Four plants, A_5–A_8, inoculated on July 18th and on a single leaf all showed the symptoms systemically. Among four other plants, R_9–R_{12}, inoculated similarly on July 24th, three showed systemic infection, and in one there was a slight delay on the side away from the inoculation. Four large plants similarly treated by Dr Henderson Smith in October showed symptoms quite systemically. Out of

sixteen plants, therefore, one showed a delay of 4 days in the appearance of the symptoms on the further side as compared with the nearer, and one case had a doubtful delay of 1–2 days. The others were characterised by systemic infection. When it is borne in mind that the time elapsing before the appearance of symptoms is at all times rather variable, and may vary from 4–18 days under not dissimilar circumstances, the slight delay found in these cases may hardly be considered as significant.

Having been unable to demonstrate definitely the localisation of the movement of the virus to the side on which inoculation was made, another type of experiment was set up.

Effect of "ringing" the stem.

The results obtained by Baur (Davis (7)) and by Bennett (2) suggested that the effect of "ringing" was to stop the movement of the infective agent. This did not appear to be so in the case of tomato, as in all of the four tomato plants tried symptoms did appear on the parts of the plants on the non-inoculated side of the "ring." In this plant regeneration takes place very quickly—the stelar tissue is essentially parenchymatous in nature. (There is further a feebly developed intraxylary phloem in the tomato.) As a consequence of this it was difficult to decide whether the symptoms appeared as a result of infection by a virus which had moved very slowly through the living cells in the xylem and the pith, or else had travelled through the phloem tissue which arose as a result of the regeneration of the tissues.

Experiments with partially killed stems.

As it was not possible to rely on the non-recovery of the "ringed" stem, an attempt was made to remove the living cells in the stem by killing them. The agent used was chloroform, which destroys the protoplasm of the parenchymatous cells and which has, apparently, but little effect on the virus of aucuba mosaic (see Henderson Smith (15)).

A region in an internode about the middle of a fairly large stem some 40 cm. high was killed over a distance of 5 cm. This was done by making two incisions at right angles through the stem with a sharp scalpel. The incisions were 1–2 cm. long. Into them was put chloroform which was also applied to the outside of the stem. The plant was first staked up and lightly tied to the stake. After some hours the outside of the treated portion of the stem was smeared with vaseline. The effect of the treatment with chloroform was seen rapidly. The tissues almost immediately lost their turgidity and at first looked as if they were waterlogged. By the

next day the treated area and a portion on either side of it had become brown and quite dead. On that day the plants were inoculated either above or below the lesion. In other cases the stem treated was that of a branch, and the distal end of the branch was treated as being "above" the lesion.

In these plants which had been so treated, the effect of the killing of the living tissue of the stem early became apparent. Adventitious roots appeared above the lesion, especially on the region immediately above the killed tissues. The appearance of one such plant is illustrated in Plate XXXIII, fig. 1. The results are given below in tabular form. Four experiments were carried out with branches treated and twenty-two with stems. In some cases the inoculation was made "above" the lesion, and in others "below." The plants were all inoculated between July 1st and July 24th, 1929.

Table II.

Details of plants inoculated after the stem had been treated with chloroform.

Tota number of treated plants (July 1st–24th, 1929)	26
No. of plants when infection did not pass lesion	14
No. of plants when infection was not delayed by lesion	4
No. of plants where tissues partially recovered	4
No. of plants where symptoms appeared across lesion	4

It can be seen in this table that fourteen plants showed no symptoms on the opposite side of the lesion. In twelve plants symptoms did appear across the lesion. There is good evidence to account for this result in the case of eight of the plants. In the four plants where "the infection was not delayed by lesion" the symptoms appeared simultaneously on either side of the lesion. It was, therefore, presumed that the "killing" of the tissues had not been quite complete and that some living cells had remained. In the four plants of the next section there was regeneration of the tissues secondarily, so that long after the symptoms had appeared on one side of the "killed" tissue the infective agent was able to move across the regenerated tissue, and to cause the appearance of symptoms on the opposite side. In the last four plants the symptoms appeared after a delay of 4–5 days and the tissue had not regenerated. It is suggested that these plants were probably accidentally infected by handling.

The whole experiment was repeated on August 23rd and 28th and September 4th–5th, 1929. The plants in this case were not now treated with chloroform, but the stems were treated with steam in a fashion similar to the chloroform treatment. A piece of damp cotton-wool was

wrapped round the stem at the middle of a suitable internode. This cotton-wool in turn was held in place by a small sheet of tin-foil wrapped round it. The outside of the tin-foil was heated with a small gas jet and the water on the cotton-wool boiled. Care was taken to protect the leaves above and below the treated internode. After this treatment the plants were left for one day, after which the outside of the dead area was vaselined. The stems in these plants again were tied to canes to prevent their falling. Inoculation was made below the killed tissues in each case.

In the first three plants, *S* 21–*S* 23, symptoms appeared below the lesions in 5 days. In *S* 24–*S* 26 they appeared in 8 days. In the plants *S* 30–*S* 44 the symptoms appeared between the 5th and the 7th day after inoculation. Untreated controls showed symptoms on the 5th day. Only one out of these plants showed symptoms above the killed area.

Table III.

Data relating to the plants with "steamed" stem.

Total number of plants	21
No. of plants showing no symptom above lesion	20
No. of plants showing symptoms above lesion	1

The plants which were used for the experiments had, unfortunately, to be tested for latent virus by being kept for 4 weeks or so. It was not possible to keep the portions above the lesions turgid for as long a time, and usually the leaves tended to wilt about the 10th day after treatment. For this reason, it was necessary to remove the upper portion of the plant just above the killed area at the end of a fortnight and to grow it separately as a cutting. The cuttings were then examined daily for symptoms.

That the xylem water current had not been cut off by the treatment received was indicated by one or two observations. (*a*) The upper portion of the treated plants remained quite turgid for over a week, usually for a fortnight. (*b*) The xylem vessels appeared quite open on sectioning the tissue. (*c*) The shoots enlongated after treatment and appeared quite normal. In the case of one of the treated branches measurements were made on two of the leaves distal to the killed tissue. The figures are given below:

Table IV.

Leaf	Length on July 3rd (mm.)	Length on July 10th (mm.)
A	22	37
B	35	60

It will be seen that in one week the increase was nearly 100 per cent. Another and probably more satisfactory method of determining if water were passing up the stem across the dead area was the following. The stem of a treated plant was removed just above the roots and the cut end immersed in a solution of eosin, or some other dye. The solution travelled up the stem and passed across the treated area without obvious delay. Similar results were obtained when the dye was absorbed at the cut end of a petiole either above or below the lesion. The solution passed from below upwards or from above downwards quite readily—the dead tissues offering no apparent obstacle to movement. The movement of Chinese ink has taken place up to 3 weeks after the killing of the tissues.

MOVEMENT OF PARTICULATE SUBSTANCES THROUGH KILLED STEMS.

Of special interest in this connection are the experiments with diluted Chinese ink and nigrosin in water. When the cut stem was placed in either of these substances, the movement through the killed area of the stem could readily be watched by noting the blackening of the vascular bundles. The movement of these materials was not stopped at the top of the lesion but, on sectioning, particles of them were found in the vascular elements of the distal portion. This proves that the vessels were not plugged with protein materials, which might have acted as colloidal filters preventing the free movement of a virus body while allowing the possibly smaller eosin molecules to pass across.

Snow (16) has found that very similar treatment in the case of bean seedlings killed the living tissues but did not, at first, impede the movement of water. He found that the distal portion of plants in which an area of stem had been killed remained turgid for some 3 weeks.

The upper portion of the plant rooted readily as a cutting, and showed symptoms comparatively rapidly after being established if it had been previously infected. Those from treated stems were grown as plants for 4 weeks before being considered as clean. The cutting was always taken just above the dead tissue.

To ensure that the time (14 days) which elapsed between inoculation and wilting was sufficient to admit of the movement of virus up a normal stem, various untreated plants were inoculated. In some of these cases the minimal dose was given—inoculations being made on a single pinna at the base of the plant. After 4, 5 or 6 days the tops were removed some five or six internodes above the inoculation. Only one of the 4-day plants did not show symptoms on the upper portion which had been treated as a cutting.

Further, controls of the same size as the treated plants, inoculated at the same time but without steamed tissue, were arranged. In every case symptoms appeared, at the top of the controls, before the distal portion of the treated plants had wilted.

The mere fact of using the tops as cuttings to test if the virus were present in them is not in itself sufficient to inhibit the development of symptoms in infected material. It has been pointed out that infected stems will root and show symptoms comparatively rapidly. If young leaves are allowed to root on sand, it has been found by the writer that subsequent inoculation on one pinna results in the appearance of virus symptoms over the whole leaf. Purdy(13) found similar results with tobacco leaves. To ensure, further, that no top was a "carrier," the leaves of some of the tops were removed at different stages and inoculated, as above described, into batches of four young actively growing plants. In no instance did the test plants develop symptoms. Incidentally, no case of a "carrier" for aucuba mosaic has yet appeared in our cultures.

To determine if any virus had passed into the dead tissue and had there been adsorbed, the following inoculations were made. The region of dead tissue was divided into three so that the middle portion was quite free from the living tissue above or below. The three portions were approximately equal. Inoculations of each of these three portions were made into sets of plants. In the first case, the lower third, all the plants developed symptoms, but in the other two sets all the plants were clean.

Possible adsorption of the virus agent.

One possibility suggested itself in the matter of the non-crossing of the killed area. It was thought that the actual killing of the tissues might have liberated some substance which inhibited the development of symptoms above the lesion, or, alternatively, the dead tissue might have absorbed the virus agent and prevented its further passage. To ascertain if either of these suggestions were probable, the following set of experiments was carried out.

A quantity of macerated leaf-tissue from virus plants was divided into three portions. One portion was inoculated directly into the first set of plants. These served as controls. The second portion was centrifuged at a high speed for 5 minutes; then thoroughly shaken up; again centrifuged for 10 minutes and thoroughly shaken. After having been centrifuged for another 10-minute period the supernatant liquid was decanted. The residue was mixed with a little sterilised distilled water. The super-

natant liquid was a clear brown colour. Sets of plants were inoculated with the supernatant liquid and with the residual material.

The third portion of the infective material was passed through muslin to remove the larger pieces of tissue. To the filtrate was added a quantity of stem tissue of uninfected plants which had been steamed in the manner previously described. The whole was carefully mixed together. The mixture was treated in exactly the same way as was the green tissue. It was alternately mixed and centrifuged. Thereafter the supernatant and the residual material were inoculated separately into sets of plants. There were, therefore, five sets of plants, viz.

(*a*) Controls inoculated with untreated macerated material.

(*b*) Plants inoculated with supernatant liquid of centrifuged macerated material.

(*c*) Plants inoculated with residue of centrifuged macerated material.

(*d*) Plants inoculated with supernatant liquid of centrifuged mixture of infected juice and boiled tissue.

(*e*) Plants inoculated with residue of centrifuged mixture of infected juice and boiled tissue.

These plants were set up on October 16th, 1929. Most of them showed symptoms of aucuba mosaic by October 29th. All were definitely infected when they were discarded on November 29th. The effect of the boiled tissue, therefore, was not appreciable under the conditions obtaining in these experiments.

DISCUSSION.

From the results of the experiments detailed above, it is evident that one point has been established regarding the movement of the virus of aucuba mosaic in tomato. It has been clearly demonstrated that the virus agent did not travel across tissue of which the living elements have been killed. Through this tissue, on the other hand, water could and did pass. The xylem vessels were not blocked, and coloured solutions passed freely upwards or downwards. No adsorption or inhibition of the causative agent could be demonstrated. One is, therefore, forced to the conclusion that the virus agent cannot travel mechanically in the xylem stream, but can only pass through the living tissue. For some reason, as yet not understood, survival of the tissue is necessary for the movement of the virus (cf. Holmes(9)). This is of more than passing interest, when it is remembered that the virus of tobacco, for example, can withstand the curing processes. In my own experiments, I have found that the

dead, dry leaves of aucuba infected plants continued to be infective when inoculated into fresh plants.

The conclusion is in complete agreement with that of Baur and of Bennett. It does not confirm the implied suggestion of Storey, who pointed out that it was not surprising that the virus travelled across a cut lamina, since the opposite side was still turgid. The implication is that, if the water passed across the leaf, the virus should also have passed. This is not necessarily so. Auchter has demonstrated, and I have confirmed his results, that there is distinct localisation in the movement of salts, etc., in the plant. It appears that, in the main, rapid lateral movement in plant tissues is confined almost entirely to water; even water-soluble salts do not appear to travel so rapidly.

It may, therefore, be stated definitely that, for the virus principles which have been studied, there is no direct evidence for movement in the xylem. All the experiments have, so far, shown that movement does not take place through the xylem tissues.

Movement in the phloem.

Movement through the phloem is more difficult of demonstration. The evidence adduced on this point must, in the nature of things, be circumstantial. It is not practicable to isolate "phloem" from "living" tissues. Two lines of argument may be pursued. If the movement were necessarily confined to the phloem tissues, there would be, it is suggested, some evidence of localisation of the symptoms to one particular portion of the plant. The leaves on the axillary shoot of the inoculated leaf and those directly above and below would, presumably, show symptoms first. The leaves on the alternate sides, that is, those with petioles more or less at right angles to the treated leaf, would next develop symptoms. The basis of this argument and the data concerned are contained in the papers on the movement of materials in plants (Caldwell(5)). The leaves on the side opposite to the treated leaf should not develop symptoms until long after the others. If movement were extremely rapid this might not necessarily hold. It has been shown, however, that it is not.

In the experiments recorded above, only one somewhat doubtful case occurred in which symptoms appeared some few days later on the opposite side. Even if this were a valid instance it is isolated, and, in any case, the difference in time is much too small to have any great value. The general conclusion to be derived from the experiments here recorded and from numerous others is that in the tomato, at least, there is no evidence whatsoever for localisation of movement to any sector of the plant. Severin

actually reports cases where the virus had, apparently, passed down the petiole of a leaf, had crossed the developing "bulb" of a beet, and passed up the petiole of the opposite leaf. The whole operation, including, presumably, some multiplication in the tissues of the opposite leaf, had occupied 2 days. This suggests, in the light of the work referred to above, that movement must have taken place across tissues other than phloem.

The rates at which movement takes place in the tissues are of value in this connection. Storey and Severin have measured movement within a petiole or within a leaf-blade. The data they report are given in the following table.

Table V.

Rate of movement of the virus of sugar beet and of maize.

Time (hr.)	Virus	Plant	Distance travelled (cm.)	Author
½	Curly-top	Beet	17·5	Severin
½	„	„	8·1	„
1	„	„	8·75 (twice)	„
1	„	„	11·8	„
1	„	„	13·1	„
1	Mosaic	Maize	10 (thrice)	Storey
2	„	„	40 „	„

In Table I Böning's data for tomato mosaic and for "streak" have already been given. In my experiments rates of the same order of magnitude were obtained, though there is always some variation due to individual differences in the plants used. There is no reason to assume from these data that the agent was moving in the xylem: rather there is strong evidence that it was not. It is difficult to believe that the infective principle could move so slowly in the water stream. Most experiments suggest a rate of some 2 mm. per hour, following needle inoculation. Any data available suggest a very much greater rate of movement for water in the xylem tissues (cf. Sachs, etc.).

Movement in the leaf tissues appears to be very much faster. In this case the material used was inoculated into the tissues by insect-vectors. These insects are known to penetrate by means of their stylets right to the phloem tissues. It is, therefore, suggested that the few observations which give very quick rates of movement were made when fortuitously the infective insect had inoculated its virus right into the phloem of one of the larger veins and when the second insect had sucked the infective juice out of the same vein. In the majority of cases, however, movement takes place from cell to cell by some mechanism of diffusion. In cases

where the phloem is injected and not seriously upset movement is more rapid (cf. Mason and Maskell (10)). This probably accounts for the isolated cases which periodically occur in work with the tomato, where symptoms appear within 4 days of inoculation. As has before been noted the symptoms first appear in the rapidly developing apical leaves.

The following general conclusion must, therefore, be drawn as a result of a consideration of the work above, viz. that there is no evidence of movement in the xylem. Direct evidence indicates that movement does not occur there in the case of, at least, three types of virus agent. Bennett has found that the curl virus of raspberry will not travel across ringed stems. Baur obtained similar results with the infectious chlorosis of *Abutilon*. I have, in this paper, shown that the causative agent of *aucuba* mosaic of tomato does not cross regions of stem where the living tissues have been killed.

As for the extra-cambial tissues, the absence of any apparent difficulty of lateral movement of the virus agent in the plants points to there being no inability on the part of the virus to travel in any living tissue. Direct evidence from inoculation through trichomes supports this view. On the other hand movement may, on occasion, be so rapid as to indicate that it must have taken place in the phloem elements.

Summary.

In this paper the movement in the plant of the causative agent of virus disease is discussed. The relevant data in the literature are summarised.

A method is described whereby a portion of the stem in the middle of a tomato plant was killed either by chloroform or by steam. In this way the living upper and lower portions of the plant were connected by a bridge of dead tissue. It is shown that the symptoms appeared in that part of the plant in which the inoculation was made. The virus agent did not travel across the dead region.

The xylem tracts were not materially affected by this treatment, and water travelled across the region. Evidence of this is the fact that the distal portion remained turgid and sometimes continued growth for a considerable time. If the stem were removed above the ground level and put into eosin solution, this travelled readily over the dead tissue. That the vessels were not occluded by protein plugs is shown by the fact that particulate substances were carried up the xylem tracts past the dead region.

No evidence of adsorption of the virus agent to the cell remains could be adduced, so it is assumed that it was not travelling in the xylem stream.

CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 429–443).

From previous experiments, it is known that the movement of metabolites and of stains tends to be localised to the side of the plant into which they are introduced. It was found that inoculation with juice of diseased plants caused systemic infection in all the treated plants. There was no apparent localisation of movement such as would have been expected had it been taking place through the vascular system. From this, and from other evidence in the literature, it is concluded that movement takes place in the living ground tissue of the plant.

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EXPLANATION OF PLATE XXXIII

In this plant the stem at one internode had been "steamed." Subsequent to inoculation below symptoms appeared on the lower part of the plant while the upper part remained healthy.

(*Received November 21st*, 1929.)